Apidologie (1995) 26, 503-510

Original article

Allozyme variability in a central Anatolian honeybee (*Apis mellifera* L) population

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(Received 1st September 1994; accepted 10 September 1995)

Summary — Central Anatolian honeybees (*Apis mellifera* L) were electrophoretically examined at 6 enzyme loci. Four loci were polymorphic (*Est*-3, *Pgm*, *Hk*, and *Mdh*) and 2 loci were found to be monomorphic (*Pgi* and *Me*). Genotypic frequencies of enzymes met Hardy–Weinberg expectations. Low levels of genetic variability were detected and heterozygosity was calculated as 0.033 ± 0.005. Gene frequencies obtained for *Pgm*, *Est*, *Hk* and *Mdh* were compared with those of the studies done in other parts of the world, especially in neighboring countries.

central Anatolian honeybee / *Apis mellifera* L / isozyme / genetic variation / starch-gel electrophoresis

INTRODUCTION

Bodenheimer (1941) were the first to attempt to classify honeybees of Anatolia based on morphometric data for the first time. Later, Maa (1953) published a formal taxonomic classification of Anatolian honeybees based on 3 museum specimens. Adam (1983) and Ruttner (1987) suggested that there were 3 different honeybee races in Turkey: *Apis mellifera anatoliaca* in central Anatolia, *Apis mellifera caucasica* in the North East and *Apis mellifera meda* in the South East (border with Syria, Iraq and Iran).

The extensive practice of migratory beekeeping is now mixing all 3 races in Turkey. A report by the Development Foundation of Turkey (TKV) mentioned that 90% of commercial beekeepers practice migratory beekeeping (Inci, 1987). During these migrations different honeybee races can hybridize with isolated local honeybee populations.

Enzyme polymorphisms in honeybees have been studied extensively within the last 2 decades. However, no study has been conducted on enzyme polymorphism in Turkish honeybees. Enzyme polymorphisms have been useful in the classification of honeybees. The objectives of this study were to determine the extent of electrophoretic variation in 6 enzyme systems and compare enzyme polymorphisms of the central

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Anatolian honey bees to those already studied in subspecies from other regions (Badino *et al,* 1983, 1985; Sheppard and McPheron, 1986; Sheppard, 1988; Del Lama *et al,* 1990).

MATERIALS AND METHODS

Honeybee samples were collected in August and September 1992 in Bala. Bala is located in central Anatolia, 70 km south of Ankara and is 2 647 km^2 in size. It has a semi-arid climate, little vegetation and there are many small hills between villages. Villages are visited by migratory beekeepers from different provinces in early summer. Other regions are never visited by migratory beekeepers. Villagers still keep their colonies in traditional trunk hives. Thirty-nine villages were visited and apiaries from 43 different locations were sampled. In each apiary 4–6 honeybee workers were sampled from the entrance of each hive (Biasiolo and Comparini, 1990). Sample size ranged between 19–55 and the total number of individuals sampled was 1 501. They were put in small labeled boxes and kept in an ice box. The thoraces of honeybees were ground and homogenates were kept at –20°C until needed for electrophoresis. Six enzyme systems, MDH (malate dehydrogenase, EC 1.1.1.37), ME (malic enzyme, EC 1.1.1.40), PGM (phosphoglucomutase, EC 5.4.2.2 formerly EC 2.5.7.11), PGI (phosphoglucose isomerase, EC 5.3.1.9), EST (esterase, EC 3.1.1), and HK (hexokinase, EC 2.7.1.1), were studied by starch-gel electrophoresis. Three enzyme systems (EST, PGI, HK) were studied using the Tris-citrate, pH 7.0 buffer system (Shaw and Prasad, 1970; Del Lama, 1988), 2 enzyme systems (MDH and ME) were studied using the Tris-HCl, pH 8.6 buffer system (Shaw and Prasad, 1970; Smith *et al,* 1972) and PGM was studied using the Tris-EDTA-maleate-magnesium, pH 7.4 buffer system (Shaw and Prasad, 1970). Gel and sample preparation and experimental conditions have been reported in previous papers (Shaw and Prasad, 1970; Del Lama, 1988). Enzyme activity was visualized by the techniques of Harris and Hopkinson (1976). Gene frequencies, enzyme heterozygosities and population heterozygosities were calculated according to Nei (1987). The test goodness of fit of gene frequencies to Hardy–Weinberg expectations was carried out using χ^2 analysis (Sokal and Rohlf, 1981).

RESULTS

Of the 6 enzyme systems assayed with horizontal starch-gel electrophoresis, 4 were polymorphic, and 2 exhibited invariant banding patterns in Bala (Central Anatolia honeybee populations). All isozymes are designated using relative mobilities with respect to the most common isozyme used as standard (mobility 100) (table I). Populations of honeybees of Bala were found to be in Hardy–Weinberg equilibrium with respect to all polymorphic enzymes (χ^2-test, $P < 0.05$).

Pgm *locus*

Pgm exhibited 2 alleles, *Pgm-75* and *Pgm-100,* according to their relative mobilities in the present study. Del Lama *et al* (1985) first reported the presence of 3 alleles at this locus; *Pgm-F*, *Pgm-M* and *Pgm-S* in the order of decreasing electrophoretic mobility in Africanized bee populations and 2 alleles in *A m carnica* originating from Germany. The alleles found in this study probably correspond to the *Pgm-M* and *Pgm-F* described in Del Lama *et al* (1985). Meixner *et al* (1994) found 3 alleles (*Pgm-75*, *Pgm-100* and *Pgm-120*) in Kenya, of which *Pgm-120* was previously unreported. The frequency of the most common allele ranged between 0.757 and 0.986 in 30 polymorphic locations.

Est-3 *locus*

The *Est-3* locus exhibited 3 alleles, *Est-70*, *Est-100* and *Est-130* as reported previously in Czechoslovakian honeybees by Sheppard and McPheron (1986) and in Kenya (Meixner *et al,* 1994). These alleles correspond to *Est-S*, *Est-M* and *Est-F*, respectively, in *A m ligustica* (Badino *et al,* 1985) and in Greek honeybees (Badino *et al,* 1988). The frequency of the most common allele at the 8 polymorphic locations ranged between 0.921 and 0.988.

Mdh *locus*

This enzyme has 5 alleles (*Mdh-65*, *Mdh-87*, *Mdh-100*, *Mdh-116* and *Mdh-133*) in Bala, central Anatolia. Five alleles *Mdh-55*, *Mdh-65*, *Mdh-80*, *Mdh-87* and *Mdh-100* were detected by different authors in different honeybee populations (Contel *et al*, 1977; Cornuet, 1979; Gardside, 1980; Nunamaker and Wilson, 1981; Badino *et al*, 1983; Nunamaker *et al*, 1984; Sheppard and Berlocher, 1984, 1985; Badino *et al*, 1985; Sheppard and McPheron, 1986; Badino *et al*, 1988; Sheppard, 1988; Lobo *et al*, 1989; Meixner *et al*, 1994). The frequency of the most common allele ranges between 0.821 and 0.988 in the 12 locations where this enzyme is polymorphic.

Hk *locus*

Hexokinase has 4 alleles (*Hk-87*, *Hk-100*, *Hk-110* and *Hk-120*). This enzyme has been studied previously by Del Lama *et al* (1988) and they found 2 alleles (*Hk-87* and *Hk-100*) in Africanized honeybees in Brazil. This locus was also studied in Kenya by Meixner *et al* (1994), who reported the presence of 2 alleles (*Hk-83* and *Hk-100*). The frequency of the most common allele in the 14 locations where this enzyme was polymorphic ranged between 0.786 and 0.991.

Pgi *and* Me *loci*

Pgi and *Me* were invariant in the central Anatolian populations. The *Pgi* locus was studied by Badino *et al* (1983, 1985, 1988), and no genetic variability was detected. *Me* variability was reported by Sheppard and Berlocher (1984; 1985) and Sheppard and McPheron (1986). Three alleles were reported (*Me-79*, *Me-100* and *Me-106*) in *A mellifera* from Norway (Sheppard and Berlocher, 1984), Italy (Sheppard and Berlocher, 1985) and western Czechoslovakia (Sheppard and McPheron, 1986). The *Me* locus is found nearly fixed in Kenya. In one colony, however, a previously unknown allele, *Me-117*, was found (Meixner *et al*, 1994).

Heterozygosities of locations ranged between 0.004 and 0.157. Overall average heterozygosity for central Anatolia was calculated as 0.033 ± 0.005.

DISCUSSION

In this study, a population honeybees from Bala was studied extensively at 43 locations. A total of 1 501 honeybee individuals were analyzed electrophoretically. Central Anatolian honeybee populations showed a low level of genetic variability in accordance with the studies on honeybees from other countries. Among the enzyme loci studied, the *Pgm* locus was the most polymorphic. For *Hk* and *Mdh* 2, new alleles were discovered in this work.

The *Pgm* locus was studied in a number of populations by Mestriner and Contel (1972), Brueckner (1974), Contel *et al* (1977), Nunamaker (1980), Nunamaker and Wilson (1980), Badino *et al* (1983), Sheppard and Berlocher (1985), and Sylvester (1986). None of these authors reported any variability at the *Pgm* locus in honeybees. Del Lama *et al* (1985) first reported variation at *Pgm* and found that the frequency of the fast allele was 0.926 in Africanized honeybees, 0.962 in Carniolan honeybees imported from Germany, and 1.000 in *A m ligustica*. In a later study Sheppard *et al* (1991) studied Brazilian honey bees and reported that the previous 'fast' and 'medium' alleles of Del Lama corresponded to relative mobilities of 1.0 *(Pgm-100)* and 0.75 *(Pgm-75)*, respectively. In our study, the slow allele *(Pgm-75)* was the common allele and the fast allele *(Pgm-100)* was rare.

Variation in the *Est* locus was similar to that reported for Greek honeybees (Badino *et*

Table I. Allele frequencies at each location, and total mean of the allele frequencies of *Pgm*, *Est*, *Hk*, and *Mdh* loci for honey bees from 43 locations in Bala, Ankara.

Location	*Number of hives*	*Number of bees*	*PGM*		*EST*			*HK*				*MDH*				
			75	*100*	*70*	*100*	*130*	*87*	*100*	*110*	*120*	*65*	*87*	*100*	*116*	*133*
1	6	28	0.875	0.125	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
2	4	24	0.833	0.167	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
3	6	26	0.962	0.038	–	1.000	–	0.038	0.962	–	–	–	–	1.000	–	–
4	5	28	0.946	0.054	–	1.000	–	0.036	0.964	–	–	–	–	1.000	–	–
5	6	33	0.939	0.061	–	1.000	–	0.061	0.939	–	–	–	–	1.000	–	–
6	5	28	1.000	–	–	1.000	–	0.125	0.875	–	–	–	–	0.982	0.018	–
7	5	24	0.917	0.083	–	1.000	–	0.021	0.979	–	–	–	–	0.979	0.021	–
8	5	25	1.000	–	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
9	6	30	1.000	–	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
10	6	28	0.821	0.179	–	1.000	–	–	0.786	–	0.214	–	–	0.821	–	0.179
11	3	19	0.868	0.132	–	0.974	0.026	–	1.000	–	–	–	–	1.000	–	–
12	4	23	0.848	0.152	–	0.935	0.065	–	0.913	–	0.087	–	–	0.913	–	0.087
13	8	43	0.779	0.221	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
14	6	34	1.000	–	0.013	0.987	–	–	1.000	–	–	–	–	1.000	–	–
15	7	38	0.974	0.026	–	1.000	–	0.013	0.974	0.013	–	–	–	1.000	–	–
16	6	32	0.844	0.156	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
17	6	36	1.000	–	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
18	6	35	0.929	0.071	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
19	8	40	0.913	0.087	–	1.000	–	–	0.988	0.012	–	0.012	–	0.988	–	–
20	6	36	0.861	0.139	0.014	0.986	–	–	0.986	0.014	–	–	–	1.000	–	–
21	7	38	0.961	0.039	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
22	6	36	0.972	0.028	–	1.000	–	–	1.000	–	–	–	0.028	0.972	–	–

Table I. Cont.

Location	*Number of hives*	*Number of bees*	*PGM*		*EST*			*HK*				*MDH*				
			75	*100*	*70*	*100*	*130*	*87*	*100*	*110*	*120*	*65*	*87*	*100*	*116*	*133*
23	8	40	1.000	–	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
24	6	35	1.000	–	–	1.000	–	–	1.000	–	–	–	0.071	0.929	–	–
25	9	45	1.000	–	0.012	0.988	–	–	1.000	–	–	–	–	1.000	–	–
26	6	37	1.000	–	–	1.000	–	–	1.000	–	–	–	–	0.851	0.149	–
27	9	48	0.802	0.198	–	1.000	–	0.062	0.938	–	–	–	–	1.000	–	–
28	7	38	0.934	0.066	0.079	0.921	–	–	0.987	–	0.013	–	0.066	0.934	–	–
29	6	31	0.984	0.016	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
30	6	34	0.971	0.029	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
31	6	35	1.000	–	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
32	10	55	1.000	–	–	1.000	–	–	0.991	–	0.009	–	–	1.000	–	–
33	8	42	0.976	0.024	0.024	0.976	–	–	0.988	–	0.012	–	0.036	0.964	–	–
34	6	37	1.000	–	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
35	8	39	0.846	0.154	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
36	9	45	0.933	0.067	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
37	6	36	0.972	0.028	–	1.000	–	–	1.000	–	–	0.042	–	0.958	–	–
38	6	35	0.988	0.012	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
39	6	34	1.000	–	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
40	6	35	0.757	0.243	0.057	0.943	–	–	1.000	–	–	–	–	1.000	–	–
41	6	37	0.986	0.014	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
42	6	35	0.814	0.186	–	1.000	–	–	1.000	–	–	–	–	1.000	–	–
43	8	44	0.818	0.182	–	1.000	–	–	1.000	–	–	0.011	–	0.909	0.080	–
Mean gene frequency			0.931	0.069	0.006	0.993	0.001	0.008	0.985	0.001	0.006	0.002	0.005	0.982	0.007	0.004

al, 1988). In this study, *Est-70* was rare and only found at 6 locations out of 43. *Est-100* was the most frequent allele and was fixed at 35 locations. *Est-130* was the other rare allele and was found at only 2 locations. Del Lama *et al* (1990) found gene frequencies similar to the present study in Africanized honeybees and European races of *A mellifera* L. Badino *et al* (1985) found similar gene frequencies in honeybees in northern Italy, but gene frequencies in samples of western and eastern Sicily were quite different from findings of Sheppard and Berlocher (1985), Badino *et al* (1988), Del Lama *et al* (1990), and the present study. The frequency of *Est-70* decreases and the frequency of *Est-100* increases as one goes from western to eastern Sicily (Badino *et al,* 1985).

In *Hk* locus we observed 2 alleles (*Hk-110* and *Hk-120*) in addition to those observed by Del Lama *et al* (1985, 1990). The frequency of *Hk-110* ranged between 0.012 and 0.014 in the 3 locations where this allele was found. The frequency of *Hk-120* varied between values 0.009 and 0.214 at 4 locations. *Hk-100* was the most frequent allele in central Anatolian honeybees. This allele was fixed in European honeybees, whereas, in Africanized honeybees the frequency of *Hk-100* ranges between 0.348 and 0.600 (Del Lama *et al,* 1990).

We found 5 alleles at the *Mdh* locus (*Mdh-65*, *Mdh-87*, *Mdh-100*, *Mdh-116* and *Mdh-133*). Sheppard (1988) reported 3 *Mdh* alleles in US honeybee populations and they were named as *Mdh-65*, *Mdh-80* and *Mdh-100*. Sheppard and Berlocher (1985) also reported *Mdh-87* in *A m ligustica*. Cornuet (1983) demonstrated a north to south cline in gene frequencies of *Mdh-80* in *A m iberica*. In Europe, including northern Spain, the frequency of *Mdh-80* is very high and the frequency of *Mdh-80* decreases towards southern Spain. Nielsen *et al* (1994) showed a similar cline in *Mdh-80* in California and mentioned parallel clines in Europe, North America and South America. We could not detect *Mdh-80* in our study. We observed *Mdh-87* in 4 out of 43 locations. *Mdh-100* was fixed in 31 locations and variable in 12 locations. In contrast to our findings, in *A m carnica* and *A m ligustica* the frequency of *Mdh-100* was rather low, and the most common allele for these subspecies was *Mdh-65* (Sheppard and Berlocher, 1985; Sheppard, 1988; Del Lama *et al,* 1990). In Greece, the frequency of *Mdh-100* decreased going north through Peloponnesos to Macedonia, but remained high in the eastern region near the border to Turkey; in Crete this allele was fixed (Badino *et al,* 1988). Although *Mdh-100* was nearly fixed in central Anatolia, in a sample from Igneada located in northwest Turkey, like the honeybee populations in northeast Greece, the frequencies of *Mdh-100* and *Mdh-65* were 0.622 and 0.378, respectively (unpublished data). The *Mdh-116* and *Mdh-133* found in this study have not been reported previously. Badino *et al* (1985) found another fast allele, which he called *F1* in eastern Sicily and Calabria. This *F1* allele may correspond to one of the fast alleles (*Mdh-116* or *Mdh-133*) present in this study. Ndiritu *et al* (1986) reported 3 *Mdh* alleles (*Mdh-100*, *Mdh-95* and *Mdh-80*) in African honeybees from Kenya. We do not know whether there is a correspondence between the *Mdh* alleles found in Kenya and in our study. However, *Mdh-100* was the most common allele in both studies.

The *Mdh-100* gene frequency in central Anatolian honeybees is close to the gene frequencies of Africanized honeybees. The *Mdh-65* gene frequency, which is low in central Anatolian and Africanized honeybees, is common in *A m ligustica* and *A m carnica*. The *Hk* locus also shows variation in both central Anatolian and Africanized honeybees, whereas *Hk-100* is fixed in *A m ligustica* and *A m carnica*. This seems to support the hypothesis (Ruttner *et al,* 1978) on the historical biogeography of honeybees that suggests honeybees dispersed from a center situated in the north east of Africa to

the near east. However, it would be premature to speculate about the evolutionary history of honeybees based on allozyme data from a single population. Studies on geographic variation of isozymes in the honeybees Turkey on a wider scale are under way.

ACKNOWLEDGMENTS

The authors wish to thank G Eren-Kandemir who assisted during collection and preparation for the electrophoresis. We thank to M Kence and CC Bilgin for their critical advice. We would like to thank the Development Foundation of Turkey (TKV) for their help in collection of samples. We would like to thank apiaries in Bala for supplying bee material. This work was supported by Turkish Scientific Research Council (Project No TBAG/1021).

Résumé — Variabilité allozymique dans une population d'abeilles d'Anatolie centrale. Les analyses électrophorétiques des allozymes ont montré peu de variabilité génétique chez l'abeille. On a recherché la variabilité génétique de 6 systèmes enzymatiques de populations d'abeilles d'Anatolie centrale : enzyme malique *(Me),* phosphoglucomutase *(Pgm),* estérase-3 *(Est-3),* hexokinase *(Hk),* phosphogluco-isomérase *(Pgi),* malate-déshydrogénase *(Mdh).* Des électrophorèses sur gels d'amidon ont été réalisées. L'activité enzymatique a été visualisée par la technique de Harris et Hopkinson (1976). La fréquence des gènes et l'hétérozygotie ont été calculées. L'équilibre de de Hardy-Weinberg a été testé. Sur les 6 systèmes étudiés, 4 se sont montrés polymorphes *(Pgm, Est-3, Mdh, Hk).* À l'exception de *Mdh* qui a une structure dimère, tous les enzymes polymorphes sont des monomères. Tous les enzymes respectent l'équilibre de Hardy-Weinberg. L'hétérozygotie était de 0,033 ± 0,005. Les populations d'abeilles d'Anatolie centrale ont présenté un faible niveau de variabilité génétique. Les fréquences de gènes pour *Mdh* et *Hk* se situent entre les fréquences trouvées en Europe et en Afrique.

abeille d'Anatolie centrale / *Apis mellifera* L / allozyme / variabilité génétique / électrophorèse sur gels d'amidon

Zusammenfassung — Allozymvariabilität in einer Population zentralanatolischer Honigbienen (*Apis mellifera* L). Zur Bestimmung der genetischen Variabilität in einer zentralanatolischen Population von Honigbienen wurden sechs Enzymsysteme untersucht (Malat-Enzym, Phosphoglucomutase, Esterase-3, Hexokinase, Phosphogluco-isomerase, Malatdehydrogenase). Die elektrophoretische Untersuchung ergab eine sehr geringe genetische Variabilität mit einem errechneten Heterozygotiegrad von 0.033 ± 0.005. Das Isoenzymbandenmuster wurde mit Stärkegel-Elektrophorese ermittelt. Die Enzymaktivität wurde mit der Technik von Harris und Hopkinson (1976) sichtbar gemacht. Die Genfrequenzen und der Heterozygotiegrad wurden berechnet, sowie die Übereinstimmung mit den Erwartungswerten nach Hardy-Weinberg überprüft. Vier der sechs Enzymsysteme *(Pgm, Est-3, Mdh, Hk)* waren polymorph. Außer *Mdh,* das eine dimere Struktur hatte, waren alle polymorphen Enzyme monomer. Bezüglich aller vier polymorphen Enzymloci befand sich die Population im Hardy-Weinberg Gleichgewicht. Die für *Pgm, Est, Hk* und *Mdh* bestimmten Genfrequenzen wurden mit den Ergebnissen aus Untersuchungen in anderen Weltteilen, besonders aber aus benachbarten Ländern, verglichen.

zentralanatolische Honigbienen / *Apis mellifera* L / Isoenzyme / genetische Variabilität / Stärkegel-Elektrophorese

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